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PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Sjöholm et al. Confirmation No: 9039

Serial No.: 09/779,323 Group Art Unit: 1852

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For: Use of Acid Stable Protease In Animal Feed

## DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Anna-Maria Künter, do hereby state and declare that

1. I received a doctoral degree in Agricultural Sciences from the Agricultural Faculty of the Rheinische Friedrich-Wilhelms-University in Bonn, Germany. From 1988 to 1990 I had a post-doctoral position in Animal Nutrition Research of F. Hoffmann-La-Roche Ltd., Switzerland. Since 1990, I have been employed by Société Chimique Roche SA (since 2001 Roche Vitamines France S.A.S.), France. From 1990 - 1994, I was a Research Scientist, and from 1994 until the present, I have been the Group Leader for the research group Poultry Nutrition. During the last nine years, my responsibilities have involved developing different enzymes for animal feed.

2. The following experiments were carried out under my direction and supervision. In each treatment shown below, 240 chickens were sorted into twelve replicate groups of twenty chickens (six groups of males and six groups of females) and were fed for thirty-six days with the animal feed composition shown in table 1 below:

Table 1: Feed composition of the experimental diet

Ingredients (%)	Days 1-22	Days 22-36
Maize	53.70	57.70
SBM 44	38.30	35.30
Soybean oil	4.10	3.80
DL-Methionine	0.20	0.08
DCP	1.70	1.80
Limestone	0.80	0.50
Salt	0.20	0.15
Premix <sup>1</sup>	1.00	1.00
TiO <sub>2</sub> <sup>2</sup>	-	0.10
Calculated content		
Crude protein (%)	21.50	20.30
ME <sub>N</sub> (MJ/kg) <sup>3</sup>	12.60	12.60
Crude fat (%)	7.20	8.80
Lysine (%)	1.21	1.13
Methionine (%)	0.53	0.37
Methionine + Cystine (%)	0.88	0.72
Analyzed content		
Crude protein (%)	19.80	18.70
ME <sub>N</sub> (MJ/kg) <sup>4</sup>	12.40	12.40
Crude fat (%)	7.80	7.30

<sup>1</sup> Including Avatec as anticoccidial

<sup>2</sup> TiO<sub>2</sub> as indigestible marker was included in the feed for treatment in days 22-36

<sup>3</sup> Calculated with EC-equation

<sup>4</sup> Calculated with EC-equation based on analyzed nutrient content

The feed composition was treated with different amounts of a protease from *Nocardiopsis* NRRL 16282 having an amino acid sequence of SEQ ID NO: 1, or with plain water as a negative control. The protease in solid form (product concentration 154 mg enzyme protein/g) was dissolved in 800 ml of water (450 kg feed per treatment for days 1-22) or 1200 ml of water (600 kg feed per treatment for days 22-36) and sprayed onto the feed pellets. The pellets for the negative control treatment (A) were sprayed with the same amounts of pure water.

In a control treatment (A) the chickens were fed the above-identified feed composition without protease, and in three other treatments the chickens were fed the above-identified composition with the protease in different concentrations as follows (mg enzyme protein per kg feed): 6.25 mg/kg (B), 12.50 mg/kg (C), and 25.00 mg/kg (D).

3. The results of the experiments are shown in Tables 2 (Days 1-36), 3A (Days 1-22), and Table 3B (Days 22-36), wherein the results of both sexes have been pooled because no significant interaction between treatment and sex occurred. In these tables the Newman-Keuls test

has been applied to the results to indicate the statistical significance, hence the mean values within a row not sharing a common superscript are significantly different ( $p < 0.05$ ).

These results clearly demonstrate that broiler chickens have a significantly improved weight gain and significantly improved feed conversion when fed an animal feed composition comprising the *Nocardiopsis protease*. These results are surprising and unexpected.

**Table 2: Performance of Broiler Chickens**

	Control	Protease from <i>Nocardopsis</i> NRRL 18282			
Treatment	A	B	C	D	
Dose (mg EP/kg feed)	0	6.25	12.5	25.0	
Pens x birds	12 x 20	12 x 20	12 x 20	12 x 20	
Weight gain (g/bird)	2013 <sup>B</sup>	2159 <sup>A</sup>	2137 <sup>A</sup>	2189 <sup>A</sup>	
Days 1-36	± 126	± 166	± 133	± 158	
%	100.0	107.2	106.2	108.8	
Feed intake (g/bird)	3403 <sup>A</sup>	3435 <sup>A</sup>	3463 <sup>A</sup>	3403 <sup>A</sup>	
Days 1-36	± 209	± 208	± 170	± 203	
%	100.0	100.9	101.5	100.0	
Feed conversion (g feed/g gain)	1.691 <sup>A</sup>	1.584 <sup>B</sup>	1.617 <sup>B</sup>	1.556 <sup>C</sup>	
Days 1-36	± 0.036	± 0.050	± 0.036	± 0.041	
%	100.0	84.3	86.6	92.0	
Mortality (%)	3.3	2.9	2.9	4.2	

Table 3A: Performance of Broiler Chickens

	Control	Protease from <i>Nocardiopeis</i> NRRL 18282			
Treatment	A	B	C	D	
Dose (mg EP/kg feed)	0	6.25	12.5	25.0	
Pens x birds	12 x 20	12 x 20	12 x 20	12 x 20	
Weight gain (g/bird)	837 <sup>B</sup>	884 <sup>A</sup>	883 <sup>A</sup>	908 <sup>A</sup>	
Days 1-22	± 81	± 65	± 47	± 67	
%	100.0	106.9	106.6	108.6	
Feed intake (g/bird)	1202 <sup>A</sup>	1208 <sup>A</sup>	1205 <sup>A</sup>	1183 <sup>A</sup>	
Days 1-22	± 73	± 77	± 62	± 71	
%	100.0	100.3	100.3	99.3	
Feed conversion (g feed/g gain)	1.438 <sup>A</sup>	1.349 <sup>BC</sup>	1.367 <sup>B</sup>	1.316 <sup>C</sup>	
Days 1-22	± 0.052	± 0.019	± 0.056	± 0.034	
%	100.0	93.8	95.1	91.6	

Table 3B: Performance of Broiler Chickens

	Control	Protease from <i>Nocardiopeis</i> NRRL 18282			
Treatment	A	B	C	D	
Dose (mg EP/kg feed)	0	6.25	12.5	25.0	
Pens x birds	12 x 20	12 x 20	12 x 20	12 x 20	
Weight gain (g/bird)	1176 <sup>B</sup>	1284 <sup>A</sup>	1255 <sup>A</sup>	1282 <sup>A</sup>	
Days 22-36	± 72	± 106	± 94	± 97	
%	100.0	107.8	106.7	109.0	
Feed intake (g/bird)	2204 <sup>A</sup>	2232 <sup>A</sup>	2249 <sup>A</sup>	2214 <sup>A</sup>	
Days 22-36	± 143	± 138	± 116	± 142	
%	100.0	101.2	102.0	100.4	
Feed conversion (g feed/g gain)	1.875 <sup>A</sup>	1.789 <sup>BC</sup>	1.796 <sup>B</sup>	1.730 <sup>C</sup>	
Days 22-36	± 0.085	± 0.079	± 0.086	± 0.085	
%	100.0	94.3	95.8	92.3	

4. The undersigned declarant declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing thereon.

Signed this 2<sup>nd</sup> day  
of September 2003

Anna-Maria Künter

Anna-Maria Künter